Chapter 2:

Interpreting Periodicity in DNA-mediated Charge Transport across Adenine Tracts[†§]

[†] adapted from Augustyn, K. E.; Genereux, J. C.; Barton, J. K. *Angew. Chem. Int. Ed.* **2007**, *46*, 5731 and Genereux, J.C.; Augustyn, K.E.; Davis, M.L.; Shao, F.; Barton, J.K. *J. Am. Chem. Soc.* **2008**, *130*, 15150.

[§] Dr. Kate Augustyn and Ms. Molly Davis performed the Rh experiments, while Dr. Fangwei Shao performed the AQ experiments.

2.1. INTRODUCTION

The DNA π-stack has the inherent ability to act as an efficient medium for charge transport (CT).[1] Long-range DNA-mediated CT is exquisitely sensitive both to the coupling of donors and acceptors into the π-stack,[2] and to the presence of lesions, mismatches, protein-induced distortions, and other defects in the integrity of base stacking.[3] This sensitivity has been exploited in the development of novel classes of DNA-based sensing technologies[4] and might be utilized *in vivo* by transcriptional activation and DNA repair pathways.[5] To fully realize the potential of this technology, it is necessary to further understand the mechanistic underpinnings of DNA-mediated CT.

Recently, a periodic dependence on adenine tract length was observed for the fluorescence quenching of photoexcited 2-aminopurine (Ap^*) by DNA-mediated CT to guanine across the adenine tract.[6] By standardizing to a system containing the redox-inactive base inosine, the contribution to quenching solely due to CT between Ap^* and guanine was isolated. The amplitudes associated with this periodicity are substantial and greater than the observed associated errors. Non-monotonicity of CT rate vs. distance has since been observed between gold and ferrocene across methyl-substituted oligophenyleneethynylene, but that result was attributed to substantial torsional variations between polymers of different lengths, an explanation that is not adaptable to these adenine tracts[7]. Instead, we interpreted our surprising result in the context of four or five base pairs being conducive to forming a CT-active domain, leading to higher CT over an adenine tract that is an integer multiple of this number. This interpretation is consistent with the conformationally-gated character of DNA-mediated CT over long distances,[8] with evidence for delocalization of the injected hole,[9] and with evidence for a similar

delocalization length in the formation of excimers along adenine tracts.[10] A similar argument has been made to explain this result in the context of a polaron hopping model,[11] and non-monotonicity was also observed in calculations that permitted delocalization.[12]

Importantly, Ap^* fluorescence quenching is insensitive to processes that occur after the CT event, including radical trapping, incoherent hopping or back electron transfer (BET). For hole acceptors in DNA, product yields for different photooxidants scale inversely to the propensity for BET,[13] and attenuating BET, both between the hole donor and the oxidized bridge and between the hole donor and oxidized acceptor, extends the lifetime of the charge separated state.[14] While other spectroscopic investigations of CT across adenine tracts have not revealed a similar periodicity, these other studies have been performed on systems for which BET is known to be substantial[15,16] or where slow trapping allows charge equilibration after the initial CT step.[17,18] We have recently shown that for both hole and electron transport, CT efficiency is dictated in the same manner by the dynamics and structure of the intervening DNA bases.[19] If the periodicity is the result of CT-active states that serve as more efficient pathways for forward CT, then they will also mediate more efficient BET. Hence, we propose that conformations that promote forward CT also promote BET, and this BET will serve to suppress the apparent periodicity.

To test this hypothesis and determine whether this periodicity is a general property of long-range DNA-mediated CT, in this chapter we consider disparate donor-acceptor systems with varying extents of BET (**Figure 2.1**). Previously, by measuring the quantum yield of damage at double guanine sites, we ranked a series of photooxidants by

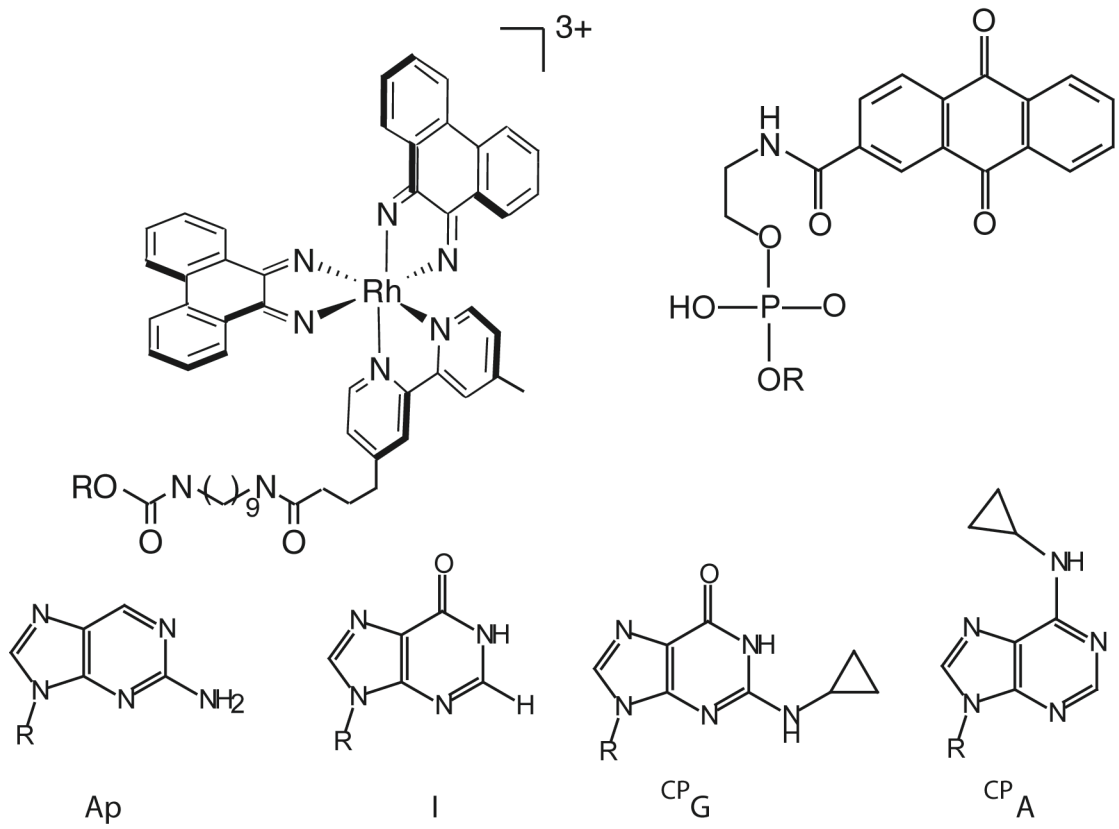

Figure 2.1. Photooxidants and modified bases used to probe CT events in DNA. At top are the structures of the rhodium and anthraquinone complexes utilized, and below are structures of aminopurine, inosine, ^{CP}G, and ^{CP}A. The rhodium complex is tethered to the 5' end of amino modified DNA by a nine carbon linker, and the anthraquinone caps the 5' end through the phosphate.

propensity for charge recombination between the guanine cation radical and the reduced hole donor.[13] Two photooxidants that are subject to only moderate BET are $Rh(phi)_2(bpy')^{3+}$ (Rh) and anthraquinone (AQ), while BET is highly efficient for Ap. Although these and other photooxidants typically induce oxidation of native guanine sites to 8-oxoguanine and further base-labile damage products,[18,20] facile BET between guanine cation radical and aminopurine anion radical renders Ap^* oxidation of guanine only observable with the ^{CP}G trap. Furthermore, to limit post-injection charge equilibration, we assay for arrival using N_2-cyclopropylguanine (^{CP}G) instead of guanine as a hole acceptor.[21] This fast[22] trap for cation and anion radicals allows detection of pre-equilibrium CT processes that are obscured by the slow trapping of guanine cation radical by water or superoxide.[26] By modulating the extent of BET for a series of ^{CP}G-containing duplexes, we demonstrate that the periodic length dependence is inherent to adenine tracts, but attenuated with increasing BET.

2.2. METHODS

2.2.1. OLIGONUCLEOTIDE SYNTHESIS

DNA oligonucleotides were synthesized trityl-on using standard phosphoramidite chemistry on an ABI DNA synthesizer with Glen Research reagents. 2-aminopurine was incorporated as the N_2-dimethylaminomethylidene-protected phosphoramidite (Glen Research). ^{CP}G-modified oligonucleotides were prepared by incorporating the precursor base, 2-fluoro-O_6-paraphenylethyl-2'-deoxyinosine (Glen Research), as a phosphoramidite at the desired position. The resin was then reacted with 1 M diaza(1,3)bicyclo[5.4.0]undecane (DBU, Aldrich) in acetonitrile to effectively remove

the O_6 protecting group. Similarly, ^{CP}A-modified oligonucleotides were prepared by incorporating the precursor base, *O*$_6$-phenylinosine (Glen Research), as a phosphoramidite at the desired position. For both ^{CP}G- and ^{CP}A- containing strands, the oligonucleotides were subsequently incubated overnight in 6 M aqueous cyclopropylamine (Aldrich) at 60 ^{o}C, resulting in substitution, base deprotection, and simultaneous cleavage from the resin. The cleaved strands were dried *in vacuo* and purified by reversed-phase HPLC, detritylated by 80% acetic acid for 15 min, and repurified by reversed-phase HPLC. Oligonucleotides were characterized by MALDI-TOF mass spectrometry.

Rhodium-modified oligonucleotides were synthesized as described previously.[27] Briefly, the detritylated resin-bound oligonucleotides were first modified with a nine carbon amine linker by reaction with carbonyldiimidazole and diaminononane in dioxane. The amine-modified strands were then reacted with $[Rh(phi)_2(bpy')]Cl_3$ (bpy' = 4-(4'-methyl-2,2'-bipyridyl) valerate) in 1:1:1 methanol:acetonitrile:isopropanol using *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyl uranium tetrafluoroborate (TSTU) as the coupling reagent. Cleavage from the resin was accomplished by incubation in NH_4OH at 60 ^{o}C for 6 hours. Strands were HPLC-purified using a Varian C_4 reversed-phase column. The two diasteromeric conjugates, differing in configuration at the metal center, have different retention times. However, both isomers were collected together and used for subsequent experiments. MALDI-TOF mass spectrometry was used to characterize the metallated DNA conjugates.

Anthraquinone (AQ)-tethered oligonucleotides were synthesized as described previously by incorporating an anthraquinone phosphoramidite at the 5´-end of the

oligonucleotides.[28] The DNA was deprotected in NH_4OH at 60 ^oC overnight. The resulting oligonucleotides were purified once by reversed-phase HPLC and characterized by MALDI-TOF mass spectrometry.

All oligonucleotides were suspended in a buffer containing 50 mM NaCl, 20 mM or 5 mM sodium phosphate, pH 7 and quantified using UV-visible spectroscopy. Duplexes were prepared by heating equal concentrations of complementary strands to 90 ^oC for 5 min and slow cooling to ambient temperature. Melting temperatures (T_m) were obtained for all duplexes. All duplexes melted between 50 ^oC and 60 ^oC at a 1.5 μM concentration in phosphate buffer (PBS, 20 mM sodium phosphate, 50 mM NaCl, pH 7).

2.2.2. PHOTOOXIDATION EXPERIMENTS

Photooxidations of Rh-tethered oligonucleotides were carried out by irradiating 30 μL aliquots of 10 μM duplex in PBS for 30 sec at 365 nm on a 1000 W Hg/Xe lamp equipped with a 320 nm long pass filter and monochromator. AQ-containing duplexes in PBS (30 μL, 10 μM) were irradiated at 350 nm using the same apparatus for 5 min. Irradiation times were varied and the decomposition was linear over the times used (supplementary information). Samples were irradiated at various temperatures ranging from 20 to 80 ^oC. Ap-containing duplexes (30 μL, 10 μM) in PBS were irradiated as above at 325 nm without the long pass filter for 30 sec or 30 min.

To analyze for ^{CP}A or ^{CP}G decomposition following irradiation, samples were digested to the component nucleosides by phosphodiesterase I (USB) and alkaline phosphatase (Roche) at 37 ^oC, to completion. The resulting deoxynucleosides were analyzed by reversed-phase HPLC using a Chemcobond 5-ODS-H, 4.6 mm × 100 mm

column. The amount of ^{CP}G per duplex was determined by taking the ratio of the area of the HPLC peak for d^{CP}G to the area of the peak for dT. The amount of ^{CP}A per duplex was determined in the same manner, using dI as the internal reference. For 30 minute irradiations, a small amount of thymine decomposition was observed, as has been described previously.[29] Hence, redox-inactive deoxyinosine was used as the internal standard for these experiments as well. The decomposition yield is taken as the percent loss of ^{CP}G or ^{CP}A between an irradiated sample and the dark control. Dark control HPLC traces were confirmed to yield the correct relative amounts of dA, dC, dG, dI, dT, d^{CP}A and d^{CP}G based on duplex sequence. Irradiations were performed at least three times and the results averaged. Due to the long irradiation times used for the Ap–I_3A_n strands, actinometry was performed using a 6 mM ferrioxalate standard[30] to allow comparison between experiments performed on separate days. The given quantum yield is for the efficiency from the Ap* state to the ring-opened product. Fluorescence quenching for the Ap–I_3A_n was not expected to be observable based on the quantum yield of ^{CP}G damage, and hence was not explored.

For ^{CP}G decomposition, errors are presented at 90% standard error of the mean, using the Student's t-distribution at the appropriate degrees of freedom to determine confidence intervals. For ^{CP}A decomposition, three trials were performed and data are reported with 2 standard errors for a 95% confidence level.

Table 2.1. DNA assemblies for oxidative decomposition experiments

Rh–A_2	3′-TICTI-AA-G^{CP}GTCTAATAACTG-5′ 5′-Rh-ACIAC-TT-C CAGATTATTGAC-3′
Rh–A_4	3′-TICTI-AAAA-G^{CP}GTCTAATCTG-5′ 5′-Rh-ACIAC-TTTT-C CAGATTAGAC-3′
Rh–A_6	3′-TICTI-AAAAAA-G^{CP}GTCTTCTG-5′ 5′-Rh-ACIAC-TTTTTT-C CAGAAGAC-3′
Rh–A_8	3′-TICTI-AAAAAAAA-G^{CP}GTCTTG-5′ 5′-Rh-ACIAC-TTTTTTTT-C CAGAAC-3′
Rh–A_8′	3′-TICTI-AAAAAAAA-G^{CP}GTCTCTATCTTG-5′ 5′-Rh-ACIAC-TTTTTTTT-C CAGAGATAGAAC-3′
Rh–A_{10}	3′-TICTI-AAAAAAAAAA-G^{CP}GTCTATCTTG-5′ 5′-Rh-ACIAC-TTTTTTTTTT-C CAGATAGAAC-3′
Rh–A_{12}	3′-TICTI-AAAAAAAAAAAA-G^{CP}GTCTCTTG-5′ 5′-Rh-ACIAC-TTTTTTTTTTTT-C CAGAGAAC-3′
AQ–A_{14}	3′-TICTI-AAAAAAAAAAAAAA-G^{CP}GTCTTG-5′ 5′-AQ-ACIAC-TTTTTTTTTTTTTT-C CAGAAC-3′
AQ–A_2	3′-TICTI-AA-G^{CP}GTCTAATAACTG-5′ 5′-AQ-ACIAC-TT-C CAGATTATTGAC-3′
AQ–A_4	3′-TICTI-AAAA-G^{CP}GTCTAATCTG-5′ 5′-AQ-ACIAC-TTTT-C CAGATTAGAC-3′
AQ–A_6	3′-TICTI-AAAAAA-G^{CP}GTCTTCTG-5′ 5′-AQ-ACIAC-TTTTTT-C CAGAAGAC-3′
AQ–A_8	3′-TICTI-AAAAAAAA-G^{CP}GTCTTG-5′ 5′-AQ-ACIAC-TTTTTTTT-C CAGAAC-3′
AQ–A_8′	3′-TICTI-AAAAAAAA-G^{CP}GTCTCTATCTTG-5′ 5′-AQ-ACIAC-TTTTTTTT-C CAGAGATAGAAC-3′
AQ–A_{10}	3′-TICTI-AAAAAAAAAA-G^{CP}GTCTATCTTG-5′ 5′-AQ-ACIAC-TTTTTTTTTT-C CAGATAGAAC-3′

AQ-A_{12} 3′-TICTI-AAAAAAAAAAAA-G^{CP}GTCTCTTG-5′
5′-AQ-ACIAC-TTTTTTTTTTTT-C CAGAGAAC-3′

AQ-A_{14} 3′-TICTI-AAAAAAAAAAAAAA-G^{CP}GTCTTG-5′
5′-AQ-ACIAC-TTTTTTTTTTTTTT-C CAGAAC-3′

Rh-A_4-$^{CP}A_2$ 3′-TICTI-^{CP}AAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC- TTTT-CCAGATTAGAC-3′

Rh-A_4-$^{CP}A_2$ 3′-TICTI-A^{CP}AAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-T TTT-CCAGATTAGAC-3′

Rh-A_4-$^{CP}A_3$ 3′-TICTI-AA^{CP}AA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TT TT-CCAGATTAGAC-3′

Rh-A_4-$^{CP}A_4$ 3′-TICTI-AAA^{CP}A-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTT T-CCAGATTAGAC-3′

Rh-A_6-$^{CP}A_1$ 3′-TICTI-^{CP}AAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC- TTTTTT-CCAGATTAGAC-3′

Rh-A_6-$^{CP}A_2$ 3′-TICTI-A^{CP}AAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-T TTTTT-CCAGATTAGAC-3′

Rh-A_5-$^{CP}A_3$ 3′-TICTI-AA^{CP}AAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TT TTTT-CCAGATTAGAC-3′

Rh-A_5-$^{CP}A_4$ 3′-TICTI-AAA^{CP}AAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTT TTT-CCAGATTAGAC-3′

Rh-A_5-$^{CP}A_5$ 3′-TICTI-AAAA^{CP}AA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTT TT-CCAGATTAGAC-3′

Rh-A_5-$^{CP}A_6$ 3′-TICTI-AAAAA^{CP}A-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTT T-CCAGATTAGAC-3′

Rh-A_{14}-$^{CP}A_1$ 3′-TICTI-^{CP}AAAAAAAAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC- TTTTTTTTTTTTTT-CCAGATTAGAC-3′

Rh-A_{14}-$^{CP}A_2$ 3′-TICTI-A^{CP}AAAAAAAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-T TTTTTTTTTTTTT-CCAGATTAGAC-3′

Rh-A_{14}-$^{CP}A_3$ 3′-TICTI-AA^{CP}AAAAAAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TT TTTTTTTTTTTT-CCAGATTAGAC-3′

Rh-A_{14}-$^{CP}A_4$ 3′-TICTI-AAA^{CP}AAAAAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTT TTTTTTTTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_5$ 3′-TICTI-AAAA^{CP}AAAAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTT TTTTTTTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_6$ 3′-TICTI-AAAAA^{CP}AAAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTT TTTTTTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_7$ 3′-TICTI-AAAAAA^{CP}AAAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTT TTTTTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_8$ 3′-TICTI-AAAAAAA^{CP}AAAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTT TTTTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_9$ 3′-TICTI-AAAAAAAA^{CP}AAAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTTT TTTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_{10}$ 3′-TICTI-AAAAAAAAA^{CP}AAAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTTTT TTTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_{11}$ 3′-TICTI-AAAAAAAAAA^{CP}AAAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTTTTT TTTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_{12}$ 3′-TICTI-AAAAAAAAAAA^{CP}AAA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTTTTT TTT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_{13}$ 3′-TICTI-AAAAAAAAAAAA^{CP}AA-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTTTTTT TT-CCAGATTAGAC-3′

Rh–A_{14}–$^{CP}A_{14}$ 3′-TICTI-AAAAAAAAAAAAA^{CP}A-GGTCTAATCTG-5′
5′-Rh-ACIAC-TTTTTTTTTTT T-CCAGATTAGAC-3′

Ap–A_2 5′-TIACTIAp-A-G^{CP}GTCTTAATCTACATCTTG-3′
3′-ACTIACT -T-C CAGAATTAGATGTAGAAC-5′

Ap–A_4 5′-TIACTIAp-AAA-G^{CP}GTCTATCTACATCTTG-3′
3′-ACTIACT -TTT-C CAGATAGATGTAGAAC-5′

Ap–A_6 5′-TIACTIAp-AAAAA-G^{CP}GTCTCTACATCTTG-3′
3′-ACTIACT -TTTTT-C CAGAGATGTAGAAC-5′

Ap–A_8 5′-TIACTIAp-AAAAAAA-G^{CP}GTCTACATCTTG-3′
3′-ACTIACT -TTTTTTT-C CAGATGTAGAAC-5′

Ap–A_{10} 5′-TIACTIAp-AAAAAAAAA-G^{CP}GTCTATCTTG-3′
3′-ACTIACT -TTTTTTTTT-C CAGATAGAAC-5′

Ap–A_{12} 5′-TIACTIAp-AAAAAAAAAAA-G^{CP}GTCTCTTG-3′
3′-ACTIACT -TTTTTTTTTTT-C CAGAGAAC-5′

Ap-A_{14}	5′-TIACTIAp-AAAAAAAAAAAAA-G^{CP}GTCTTG-3′ 3′-ACIIACT -TTTTTTTTTTTTT-C CAGAAC-5′
Ap-I_3A_2	5′-TIACTIApIII-A-G^{CP}GTCTTAATCTACATCTTG-3′ 3′-ACTIACT CCC-T-C CAGAATTAGATGTAGAAC-5′
Ap-I_3A_4	5′-TIACTIApIII-AAA-G^{CP}GTCTATCTACATCTTG-3′ 3′-ACTIACT CCC-TTT-C CAGATAGATGTAGAAC-5′
Ap-I_3A_6	5′-TIACTIApIII-AAAAA-G^{CP}GTCTCTACATCTTG-3′ 3′-ACTIACT CCC-TTTTT-C CAGAGATGTAGAAC-5′
Ap-I_3A_8	5′-TIACTIApIII-AAAAAAA-G^{CP}GTCTACATCTTG-3′ 3′-ACTIACT CCC-TTTTTTT-C CAGATGTAGAAC-5′
Ap-I_3A_{10}	5′-TIACTIApIII-AAAAAAAAA-G^{CP}GTCTATCTTG-3′ 3′-ACTIACT CCC-TTTTTTTTT-C CAGATAGAAC-5′
Ap-I_3A_{12}	5′-TIACTIApIII-AAAAAAAAAAA-G^{CP}GTCTCTTG-3′ 3′-ACTIACT CCC-TTTTTTTTTTT-C CAGAGAAC-5′
Ap-I_3A_{14}	5′-TIACTIApIII-AAAAAAAAAAAAA-G^{CP}GTCTTG-3′ 3′-ACIIACT CCC-TTTTTTTTTTTTT-C CAGAAC-5′

2.3 RESULTS

2.3.1. EXPERIMENTAL DESIGN

Figure 2.1 illustrates typical DNA-photooxidant assemblies. The Rh-A_n, AQ-A_n and Ap-A_n series contain rhodium, anthraquinone, or aminopurine separated from ^{CP}G by a bridge containing increasing numbers of adenines, while Rh-A_n-^{CP}A sequences contain ^{CP}A serially inserted along the adenine tract. For all Rh-modified assemblies there is a four base-pair segment surrounding the rhodium binding site to provide optimum intercalation of the photooxidant. Likely a mixture of binding sites (one and two bases in) are available to the rhodium diastereomers.[27] On the side distal to the hole trap, there is a constant three-base sequence so that end effects are minimized. Guanine can serve as a thermodynamic well if placed near the rhodium intercalation site and, although the trapping rate is slow, BET to rhodium is comparably fast at short distance.[13] Therefore, inosine was employed as a substitute for guanine near the rhodium binding site to enhance ^{CP}G decomposition.[9,19] Note that the first four adenine tract sequences, Rh–A_2 through Rh–A_8 are composed of 20 base pairs, while that of Rh–A_8' through Rh–A_{14} are slightly longer, with 26 base pairs (**Table 2.1**). Rh–A_8 and Rh–A_8', both containing the 8 base-pair long adenine tract but differing in length, yield equivalent decomposition profiles with both time and temperature, and in subsequent results and figures, the data from Rh-A_8' are presented. A series of HPLC traces from the time-course of AQ-A_2 degradation shows the well-resolved peaks corresponding to the six different natural and unnatural nucleosides (**Figure 2.2**).

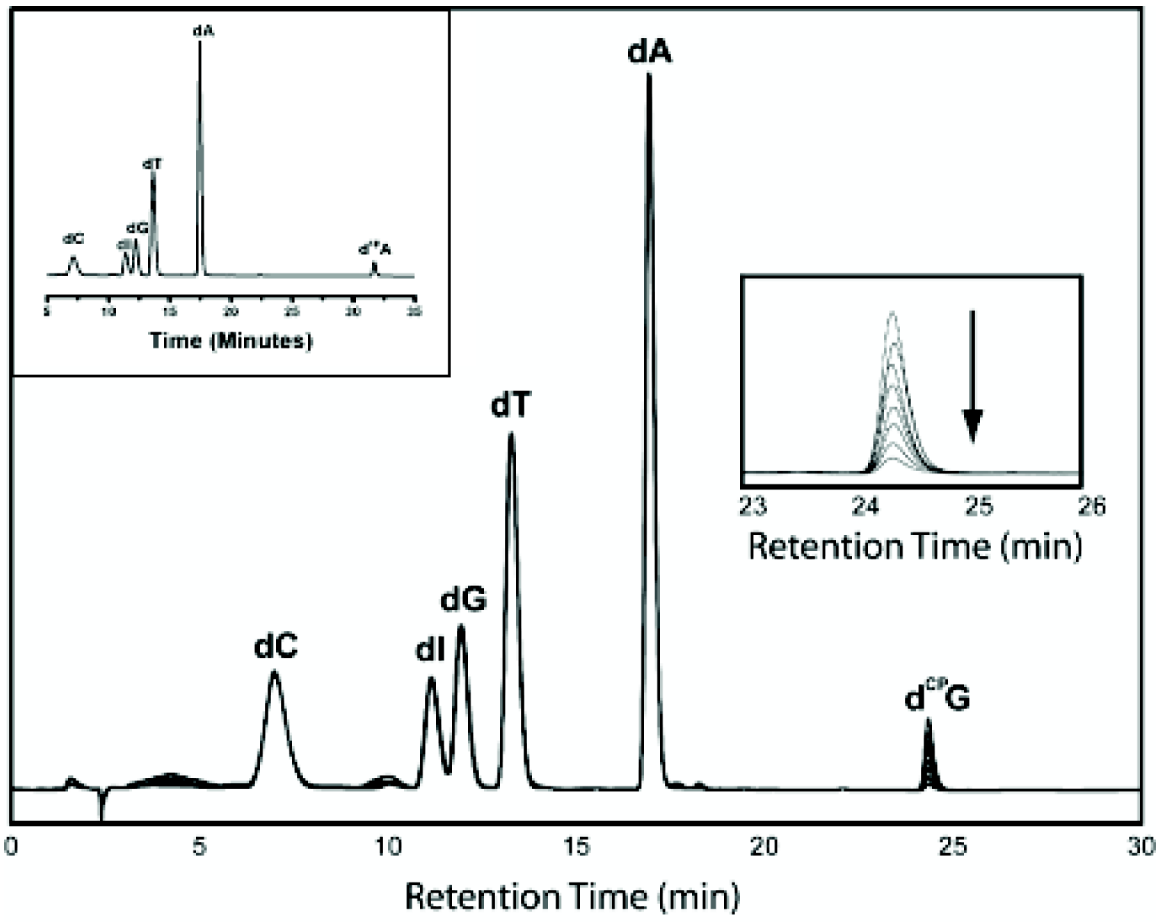

Figure 2.2. Overlaid HPLC traces at 260 nm for digested nucleosides from AQ–A_2 irradiated at 350 nm for 0, 1, 2, 3, 5, 7, 10, and 15 min. Traces are normalized to the height of the dT peak, and the righthand inset demonstrates that the peak corresponding to $d^{CP}G$ steadily degrades with respect to increased irradiation time. The lefthand inset is a chromatogram generated from digested nucleosides from a ^{CP}A-containing oligonucleotide. Conditions are as described in Methods.

2.3.2. DNA-MEDIATED OXIDATIVE DECOMPOSITION OF ^{CP}G BY RH AND AQ

Irradiation leads to the first-order decomposition of ^{CP}G by Rh and AQ, and of ^{CP}A by Rh (**Figure 2.3**). **Figure 2.4** shows the variation in the decomposition yield (Y) as a function of bridge length for the Rh-A_n and AQ-A_n series. Notably, the same non-monotonic, apparently periodic decay is observed for the Rh-A_n series as was seen for the Ap^* fluorescence quenching.[6] The apparent period of about five base pairs is similar as well, as is the temperature dependence for the Rh-A_n sequences. Below the T_m of the duplex, increasing temperature leads to increased ^{CP}G decomposition, but the amplitude of the periodicity is suppressed. Once the duplexes begin to melt, unstacking the base pairs, the decomposition efficiencies sharply drop to zero (**Figure 2.5**). This decrease in decomposition occurs between 50 and 60 ^{o}C.

Although the apparent periodicity is dampened, a similar profile is apparent with anthraquinone as the pendant photooxidant (**Figure 2.4**). As with the Rh-A_n series, photooxidation of the AQ-A_n assemblies show a shallow, non-monotonic periodic length dependence in yield. Decay parameters and apparent period are comparable.

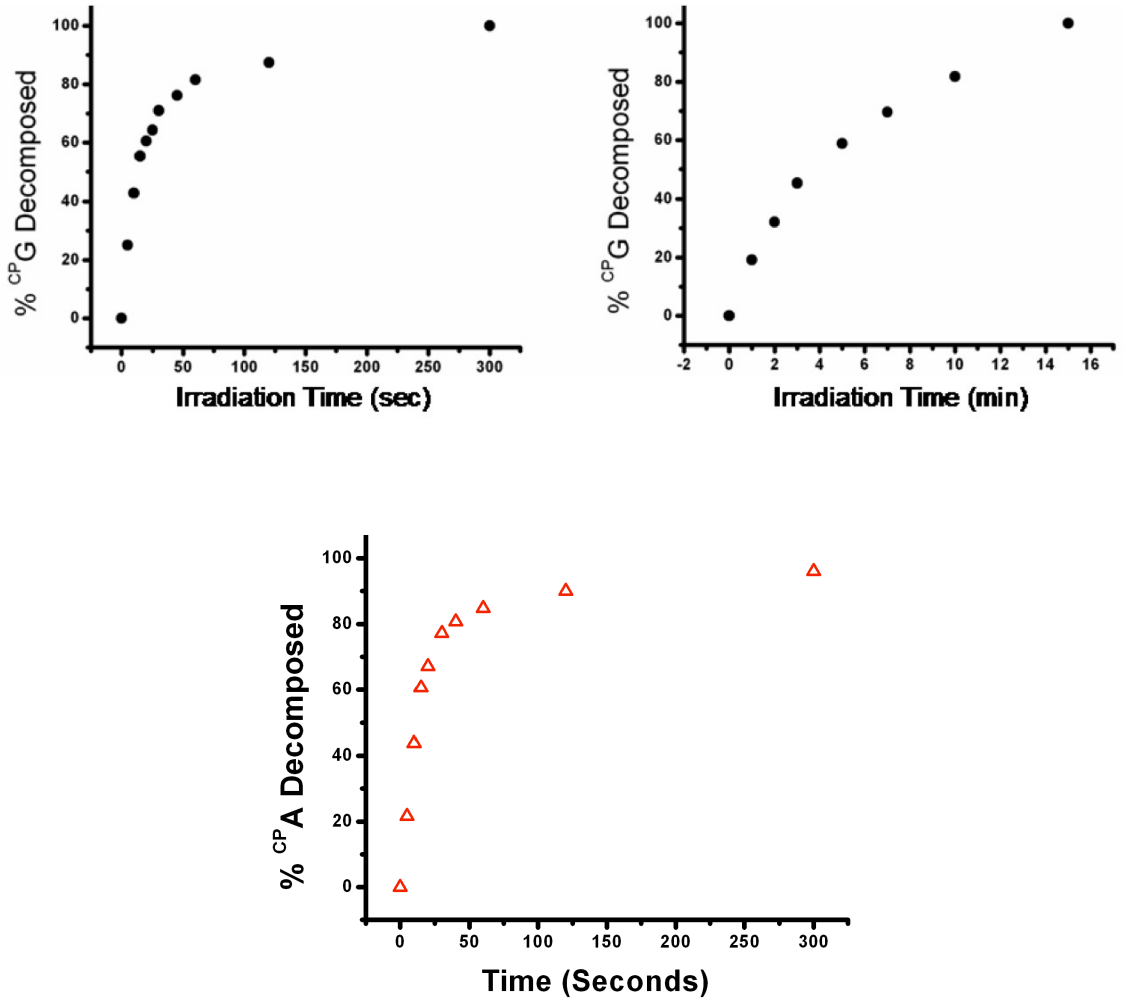

Figure 2.3. Time courses of ^{CP}G decomposition by irradiation of Rh–A_2 (top left), AQ–A_2 (top right), and Rh–A_{14}–$^{CP}A_1$. 10 μM duplexes were irradiated at 365 nm (Rh) or 350 nm (AQ). Conditions are as provided in Methods.

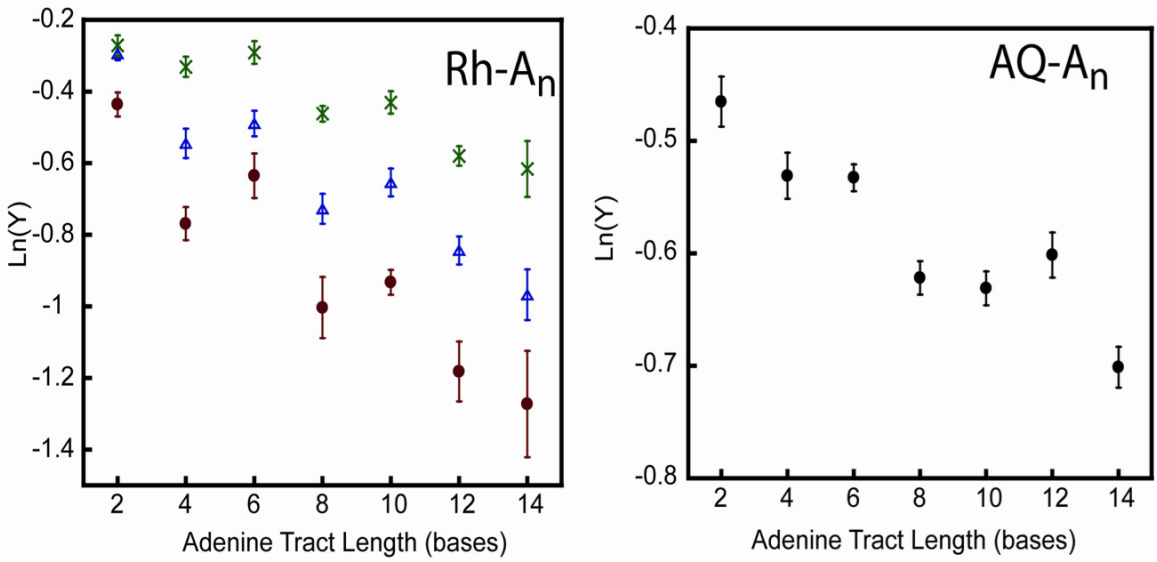

Figure 2.4. CT yields (Y) as a function of bridge length for the Rh–A_n series and AQ–A_n series. Results at three temperatures are shown for the Rh–A_n series: 20 ^{o}C (red circles), 30 ^{o}C (blue triangles), and 40 ^{o}C (green x's); AQ–A_n experiments are at ambient temperature. Duplexes (10 μM) were irradiated at 365 nm in 20 mM sodium phosphate, 50 mM NaCl, pH 7.0 as described in the text. The bridge length is defined as the number of adenines between the photooxidant and the trap. The experiments were repeated at least three times, the results averaged, and the error is expressed as 90% confidence intervals of the mean.

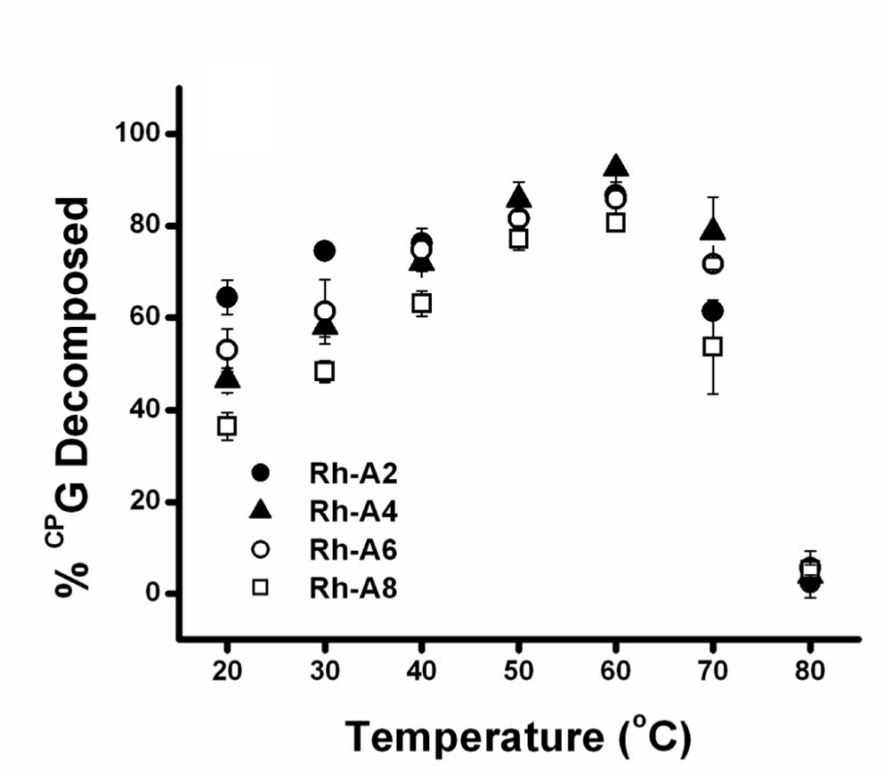

Figure 2.5. Temperature dependence for Rh–A_2 through Rh–A_8, conditions as in text. As each duplex becomes dehybridized, DNA-mediated CT is completely attenuated. Errors are single standard deviation.

2.3.3. DNA-MEDIATED OXIDATIVE DECOMPOSITION OF ^{CP}G BY AP.

To determine if periodicities could be observed in the presence of facile BET, we prepared the series of duplexes ApA_n. **Figure 2.6** directly compares the CT yield for ^{CP}G decomposition and Ap^* fluorescence quenching. Although oxidative damage to ^{CP}G is observed, ^{CP}G immediately neighboring Ap does not allow a sufficiently long-lived charge-separated state, and BET depletes the oxidized base faster than ring-opening.[13] This initial low yield for a single intervening adenine, and much higher yield for three intervening adenines, is characteristic of a system with rapid charge recombination.[14,15] Notably, although the length dependence is comparable to the fluorescence quenching result, the corresponding periodicity is completely suppressed.

To suppress BET, we tested separating the Ap from the adenine tract with a variety of higher energy sequences, including a single inosine and the sequence CTI; we found that with I_3A_n as the bridge, there is more ^{CP}G decomposition for Ap–I_3A_1 than for Ap–I_3A_3 indicating suppression of BET. For the Ap-I_3A_n sequences (**Figure 2.7**), there is substantially less damage, such that 30 min of irradiation is necessary to achieve significant decomposition of the ^{CP}G. BET is suppressed, as only slightly more decomposition is observed for the Ap-I_3A_3 sequence versus the Ap-I_3A_1 sequence. The non-monotonicity is now recovered, and is qualitatively similar to that observed for the Ap^* fluorescence quenching and Rh-A_n systems.

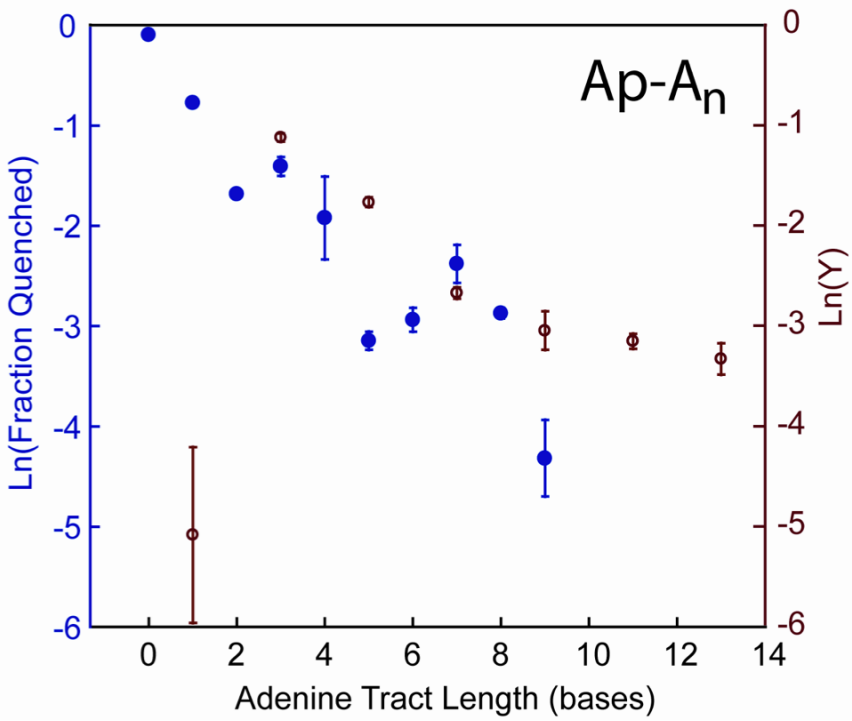

Figure 2.6. CT yields (Y) as a function of bridge length for the Ap–A_n series (red, open circles), as determined by ring-opening of ^{CP}G. Duplexes (10 μM) were irradiated at ambient temperature for 30 sec at 325 nm in 5 mM sodium phosphate, 50 mM NaCl, pH 7.0 as described in the text. The experiments were repeated at least three times, the results averaged, and the error is expressed as 90% confidence intervals of the mean. On the same plot, fluorescence quenching from reference (6) is shown for comparison (blue, closed circles).

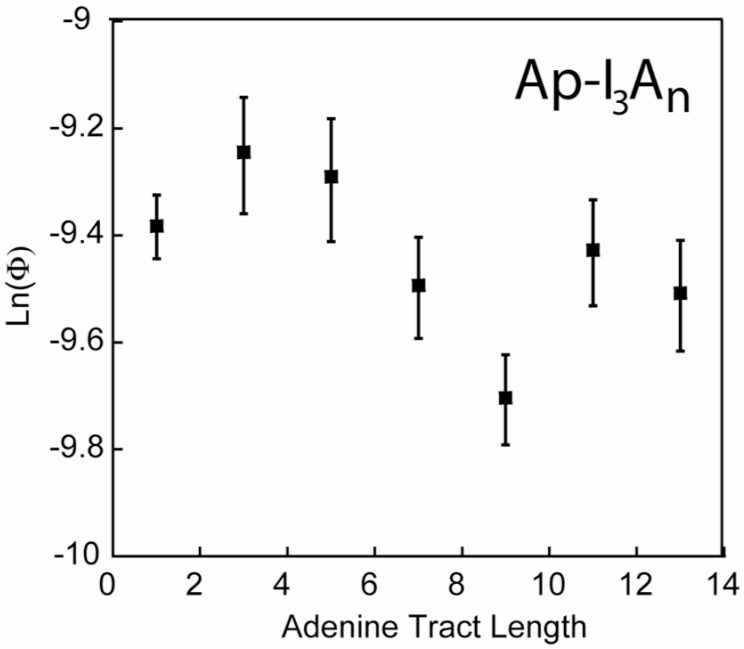

Figure 2.7. CT quantum yields (Φ) as a function of bridge length for the Ap–I_3A_n series, as determined by ring-opening of ^{CP}G. Duplexes (10 μM) were irradiated at ambient temperature for 30 min at 325 nm in 5 mM sodium phosphate, 50 mM NaCl, pH 7.0 as described in the text. The experiments were repeated at least eight times, the results averaged, and the error is expressed as 90% confidence intervals of the mean. Quantum yields were determined using actinometry on 6 mM ferrioxalate.

2.3.4. DNA-MEDIATED OXIDATIVE DECOMPOSITION OF ^{CP}A BY RH

To discriminate whether the periodicity for ^{CP}G decomposition in Rh–A_n constructs is dependent on the length of the adenine tract or on the donor-acceptor distance, we studied a series of assemblies where ^{CP}A is serially moved along the adenine tract. For these studies, we varied donor-acceptor distance while keeping the adenine tract length constant, for three sets of adenine tract lengths. Remarkably, over the 14 base pair A-tract, we find essentially no decay, with $\beta = 0.0013(3)$ Å^{-1} (**Figure 2.8**).

2.4. DISCUSSION

2.4.1. OBSERVATION OF PERIODICITIES IN LENGTH DEPENDENCE OF ^{CP}G DECOMPOSITION

The dependence of ^{CP}G oxidation by Rh or AQ on the length of the intervening adenine tract is periodic. It is striking that this result is so similar to that seen with the Ap* fluorescence quenching assay and that the periods are identical. The driving forces for photooxidation by Ap*, Rh*, and AQ* vary over a range of 700 mV.[2,31,32] The fluorescence quenching assay measures direct hole injection from Ap* into an orbital that includes the acceptor guanine, while the ^{CP}G assays directly measure the total CT yield to the hole acceptor, regardless of mechanism. Nevertheless, despite these fundamental differences between the experiments, a periodic length dependence is observed for all three cases and approximately the same apparent period is observed. Importantly, when the slow, unmodified guanine trap is used, no periodicity is observed, indicating the importance of assaying pre-equilibrium states in CT experiments. Although the ^{CP}G decomposition is a chemical event, the fast timescale of ring-opening defines a fast clock

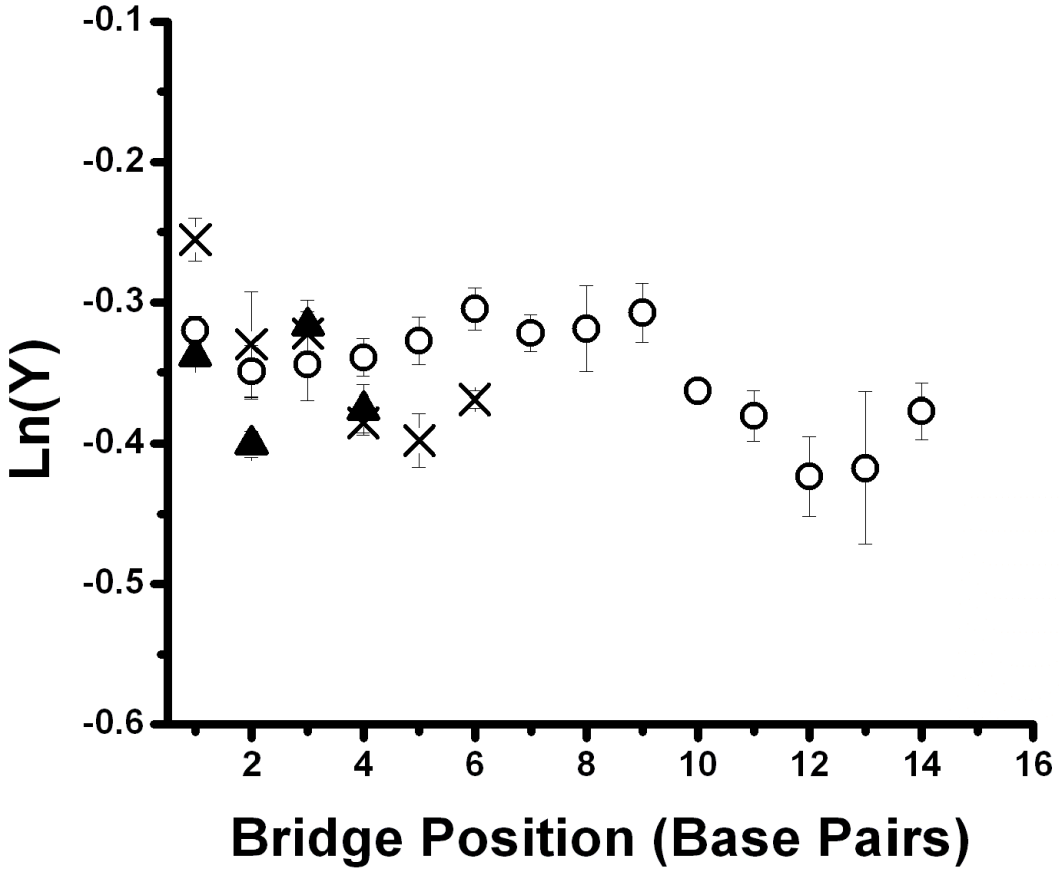

Figure 2.8. Decomposition (Y), as a function of bridge position for the Rh–A_4–$^{CP}A_m$ (closed triangles), Rh–A_6–$^{CP}A_m$ (X), and Rh–A_{14}–$^{CP}A_m$ (open circles) series following a 30 second irradiation at 365 nm. Decomposition was determined by integrating the HPLC ^{CP}A peak in an irradiated sample relative to a non-irradiated sample. Each HPLC trace was normalized to an internal inosine standard. The bars correspond to two standard errors for a 95% confidence level.

such that CT is still rate-limiting, in contrast to biochemical experiments measuring guanine decomposition.

For the Rh–A_n series, with increasing temperature, the overall yield of CT increases, the length dependence becomes shallower, and the periodicity is attenuated. For a direct CT event between a donor and acceptor in contact, in which the donor and acceptor orbitals are already aligned, higher temperatures are likely to decrease the probability that the orbitals will remain aligned, and decreased CT results. In contrast, when the donor and acceptor are separated by a dynamic bridge of base pairs, increasing the temperature allows a greater fraction of these duplexes to access a CT-active domain, resulting in enhanced CT. Increased temperature has a more prominent effect on CT through longer adenine bridges because there is a lower initial probability of each bridging base being aligned in a CT-active conformation. This effect is identical to that observed for Ap^* fluorescence quenching.[6] Furthermore, for both cases, the apparent periodicity is suppressed with increasing temperature, implying that the underlying cause of the periodicity is the same. Periodicity is not as evident for the AQ–A_n system as for the Rh–A_n sequences. This apparent decrease in amplitude could be because the AQ is separated from the adenine tract by five bases, introducing dephasing processes. Furthermore, anionic AQ can equilibrate between singlet and triplet states, the former of which is competent to reduce oxygen[34], generating a persistent hole in the DNA that can equilibrate over a long time scale and damage ^{CP}G independently of the bridging sequence, although previous work[13] has shown only a modest effect of oxygen on ^{CP}G ring-opening rates by AQ. Nevertheless, there is clear deviation from monotonicity that is

greater than experimental error, and a period equivalent in length to that observed for the Rh–A_n is evident.

In a sense, the Ap–A_n-^{CP}G sequences should represent an intermediate system between the Ap^* fluorescence quenching and ^{CP}G decomposition assays. The photooxidant is the same as in the fluorescence quenching study, and ^{CP}G decomposition is used as a proxy for charge separation, as with the Rh–A_n and AQ–A_n series. Remarkably, the decay is monotonic (**Figure 2.4**), with a decreasing slope similar to that observed in a system using stilbene as a photooxidant.[16] This could be due to a higher proportion of initial CT-active conformations for short lengths[8] or to changing distribution of yield with length between superexchange, localized hopping, and delocalized hopping mechanisms. Nevertheless, the only consistent difference between the Ap–A_n system and the other three is the presence of efficient BET. Clearly, we can control this non-monotonic effect by changing the extent of BET.

We next considered the effect of eliminating BET while still assaying for ring-opening. The timescale required for efficient charge injection is the nanosecond lifetime of Ap^*, while BET must compete with the faster ring-opening. Hence, we speculated that a bridge modification that sufficiently decreased the rate of CT in both directions could eliminate BET while still maintaining some efficiency for forward transfer.[14,35] Ap^* does not oxidize inosine, and the introduction of inosine into an adenine bridge substantially affects the CT yield. We introduced three inosines between the aminopurine and the adenine tract (**Figure 2.5**). As expected, the total CT efficiency dropped substantially, but the Ap–I_3A_1 sequence has equivalent damage yield to the Ap-I_3A_3 sequence, indicating that BET has been mostly excluded from the system. Importantly, the non-monotonicity

is now restored, supporting the hypothesis that BET was responsible for suppressing the periodicity.

These results are straightforward to reconcile with two recent studies on CT across adenine tracts. In one system, transient absorption spectroscopy was used to measure the production of NDI radical, with PTZ across an A tract participating as the hole acceptor.[15] No periodicity was observed, but it was found that BET substantially depletes the charge-separated state. Similarly, another series of experiments considered CT across an adenine tract between two capping stilbenes.[16] The length dependence found in this study is identical to that for Ap–A_n–^{CP}G, and no periodicity was observed. Furthermore, BET of the injected hole is rapid in this system as well. Notably, although a recent theoretical treatment of three-adenine tracts implied that the stiffness introduced by the bridging stilbene used in this study does not profoundly influence local coupling constants[36], this environment might well affect formation of delocalized domains.

2.4.2. Distance Dependence for RH–A_N–$^{CP}A_M$ Decomposition

The distance dependence for CT yield is dependent on the injection yield, the contribution of incoherent and coherent channels, and the relative rates of charge recombination, charge migration, and charge trapping. It is unlikely that measured effective logarithmic distance dependences of CT in DNA correspond to the inherent electronic coupling factor β, particularly for cases where hopping is the dominant mechanism.

Adenine tracts are particularly interesting as a medium for CT due to their resistance to inherent charge trapping,[15,29] structural homogeneity, and established

efficient CT.[6,15,16] Yields of CT from sugar radicals to triple guanine sites were found to decrease exponentially with increasing A-tract length up to three adenine base pairs, but yields through longer A-tracts followed a weaker distance dependence.[17] The kinetics of CT through A-tracts were examined later by transient absorption of stilbene-capped hairpins; rates with increasingly weak distance dependences were attributed to superexchange, localized hopping, and delocalized hopping with limiting $\beta \sim 0.1$ Å^{-1}.[16] Studies to examine injection yields of CT through A-tracts have also been performed with phenothiazine as the hole acceptor and naphthaldiimide as the hole donor, with $\beta = 0.08$ Å^{-1}.[15] With phenothiazine and 8-oxo guanine, a β value of 0.2 Å^{-1} is observed. Interestingly, when the A-tract is disrupted by insertion of a double guanine site, CT is attenuated. We have investigated charge injection through increasing length A-tracts by monitoring the quenching of photoexcited 2-aminopurine by guanine and also observe a shallow distance dependence ($\beta \sim 0.1$ Å^{-1}).[6]

CT over the 14 base pair A-tract is distance-independent (**Figure 2.8**), contrasting strongly with previous studies using acceptors that are external to the bridge. This is not merely a consequence of attenuated BET, as a steeper distance dependence of roughly 0.02 Å^{-1} is found for the Rh–A_n assemblies, where both the photooxidant and the injection environment are identical. The flatness implies that all holes reach the adenine tract terminus following injection. Thus, the timescale for transport over the entire 48 Å adenine tract must be faster than BET from the first bridge position. These data cannot be explained by a localized hopping mechanism through the 14 bases of the A-tract.

These results also demonstrate the importance of fast traps. Guanine damage experiments[13,17] also result in a shallow distance dependence across adenine tracts over

longer distance, but with a guanine trap there is charge equilibration prior to the millisecond trapping event.[37] Here, the cyclopropylamine ring opening occurs faster than charge equilibration. We previously found that the stacking of the donor and acceptor with the DNA bases has a dramatic effect on the distance dependence of CT through adenine tracts.[2] With ethenoadenine, a poorly stacked adenine analogue, as the photooxidant, a steeper β value of 1.0 $Å^{-1}$ is found, consistent with poorly coupled superexchange. This is a characteristic value found for purely σ bonded systems.[38] With the well-stacked adenine analogue 2-aminopurine as photooxidant, the distance dependence is that expected in well-stacked systems. In this context, the present results are not surprising.

Thus, a well-coupled trap incorporated into an A-tract bridge can be oxidized through DNA-mediated CT without significant attenuation over 5 nm. This is not due to suppression of BET, but rather to the competition between BET and oxidation of the trap being sensitive to the nature of the trap, even for large separation. These results are completely consistent with a fully delocalized transport model.

2.4.3. CONFORMATIONAL GATING THROUGH DELOCALIZED CT-ACTIVE DOMAINS

Previously, we interpreted the periodic length dependence in the context of a certain number of bases being ideal for forming a CT-active domain.[6] When an integer number of CT-active domains can readily form between the acceptor and donor, CT is accelerated, either coherently through two mutually CT-active domains or incoherently by hopping between such domains. For a non-integer number of domains, dephasing processes, such as domain drift, are required. These processes are slower and decrease the

probability of CT to the acceptor before charge recombination. A similar argument has been made in the context of polaron hopping.[11] The experiments described here do not distinguish between the two mechanistic arguments. Nevertheless, the fact that BET suppresses the periodicity supports the notion that increased CT across certain bridge lengths is the inherent source of the periodicity.

Since the conformationally gated domain hopping model ascribes the periodicity to the change in A-tract length, it is interesting to compare distance dependences to a system in which the A-tract length is fixed. This was accomplished by monitoring decomposition of cyclopropyladenine (^{CP}A) serially substituted at each position within a 14 base-pair adenine tract. In contrast to the ^{CP}G trapping situation, there is no periodic variation of the yield with ^{CP}A position for a given A-tract length. This result is consistent with our domain hopping model, as a given length A-tract will accommodate a similar domain structure regardless of the placement of the trap.

2.4.4. OTHER THEORETICAL PREDICTIONS OF PERIODIC DISTANCE DEPENDENCE

There have been theoretical predictions of a periodic length dependence of CT. In particular, when the energies of the donor, bridge, and acceptor are similar, on-resonance CT has been calculated to have a periodic length dependence.[39-41] In these theoretical studies of molecular wires, though an exponential distance dependence was found for off-resonance CT, smooth, bounded periodicities were predicted for on-resonance coupling; energetic inhomogeneities along the bridge could attenuate the periodicities.[40] Although these studies modeled the wire between metals, the same analyses could apply to a sufficiently gated charge-transfer system, such that the donor can be excited

independently of the bridge. It is possible that DNA fulfills that requirement based on the apparent conformational gating. A separate novel approach to determine the coupling across a molecular bridge formulated the lengthening of the bridge as iterative perturbations. Here, too, a non-monotonicity was predicted for on-resonant transfer, but was aperiodic and unstable with respect to the coupling parameters.[41]

Interestingly, Renger and Marcus have calculated a periodic length dependence for CT across an A-tract DNA bridge using a model that allowed delocalization of the electron hole over several bases.[12] These periodicities were eliminated by incorporation of a static disorder term.

The periodic length dependence found in this study does not appear to be related to on-resonance CT. The periods are the same for the different photooxidants, Ap, Rh, AQ, with different oxidation potentials; this similarity argues that the periodicity is not electronic in nature. More importantly, these theoretical periodicities are all with regard to donor-acceptor separation, not adenine tract length. Only the CT-active domain model predicts that serially inserting a ^{CP}A trap along an adenine tract of constant length will eliminate the periodicity; a quantum or symmetry effect would be, if anything, more pronounced in such a system.

It is remarkable that we are able to observe these periodicities in DNA CT using disparate assays so long as the experiments probe events on a fast timescale and isolate convoluting processes such as BET and trapping events. The observations here underscore the utility of applying cyclopropylamine-modified bases as fast traps for CT. More importantly, it is clear that engineering differing extents of BET allows control over the extent of length-dependent periodic behavior.

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